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Remarks

Reconsideration of this Application is respectfully requested.

Upon entry of the foregoing amendment, claims 1, 6-8 and 18-21 are pending in the application, with 1 and 18 being the independent claims. Support for these claims can be found in the following sections of the specification: page 4, lines 3 and 23; page 6, lines 24 and 28; page 9, line 3; page 10, line 13; page 11; line 9; and pages 29-30. These changes are believed to introduce no new matter, and their entry is respectfully requested.

Based on the above amendment and the following remarks, Applicants respectfully request that the Examiner reconsider all outstanding objections and rejections and that they be withdrawn.

Document AT3 on PTO-1449 Form

Applicants enclose a copy of the post card receipt date stamped on July 21, 2000, indicating receipt by the PTO of the Information Disclosure Statement with documents AR1, AS1, AT1, AR2, AS2, AT2, AR3, AS3 and AT3. For the convenience of the Examiner, Applicants submit herewith a copy of document AT3 which was not considered by the Examiner.

Applicants respectfully request that the Examiner review this document and forward a PTO-892 form to acknowledge consideration in the next Office Action.

Rejections under 35 U.S.C. § 112, First Paragraph

At pages 2-3 of the Office Action, the Examiner has rejected claims 1-13 and 18-23 under 35 U.S.C. § 112, first paragraph, as allegedly containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the

relevant art that the inventors, at the time the application was filed, had possession of the claimed invention. Applicants respectfully traverse this rejection.

Applicants have amended claims 1, 6-8 and 18-21 such that the claims are drawn to a method of producing amino acids selected from the group consisting of L-lysine, L-threonine, and L-isoleucine comprising: culturing a *Corynebacterium glutamicum* cell with a disrupted *pgi* gene. Amended claims 1, 6-8, and 18-21 are described in full, concise and exact terms that a skilled artisan would recognize that Applicants were in possession of the claimed invention. Support for these claims can be found in the following sections of the specification: page 4, lines 3 and 23; page 6, lines 24 and 28; page 9, line 3; page 10, line 13; page 11; line 9; and pages 29-30. Therefore, withdrawal of this rejection is respectfully requested.

At pages 3-5 of the Office Action, the Examiner has rejected claims 1-7 and 18-20 under 35 U.S.C. §112, first paragraph as allegedly failing to enable any person skilled in the art to which the invention pertains, or with which it is most nearly connected, to make and/or use the invention commensurate in scope with these claims. Applicants respectfully traverse this rejection.

The Examiner states "Applicants' specification is enabling for a method of producing lysine, threonine, and isoleucine comprising: culturing an altered C. glutamicum cell having a disrupted *pgi* gene . . ." Applicants have amended claim 1 such that it is directed to a method of producing amino acids selected from the group consisting of L-lysine, L-threonine, and L-isoleucine comprising culturing a *Corynebacterium glutamicum* cell with a disrupted *pgi* gene. Therefore, withdrawal of this rejection is respectfully requested.

Rejections under 35 U.S.C. § 103

At pages 5-7 of the Office Action, the Examiner has rejected claims 1, 6, 7 and 18-20 under 35 U.S.C. § 103(a) as allegedly unpatentable over Mascarenhas *et al.*, *Appl. Environ. Microbiol. 57*:2995-2999 (1991) ("Mascarenhas") in view of Ishino *et al.*, *J. Gen. Appl. Microbiol. 37*:157-165 (1991) ("Ishino"), Voet *et al.*, Biochemistry 2nd Ed., Wiley and Sons, NY (1995) ("Voet") and Sahm *et al.*, *Ann. NY Acad. Sci. 782*:25-39 ("Sahm"). Applicants respectfully traverse this rejection.

Mascarenhas *et al.* suggest only that the deletion of the *pgi* gene may have an effect on the biosynthetic capabilities of *Escherichia coli* for the production of aromatic intermediates.

At page 7 of the Office Action the Examiner states that "one of ordinary skill in the art would have a reasonable expectation of increased amino acid yields by disruption of the *C. glutamicum pgi* gene." Applicants respectfully disagree with the Examiner.

The Examiner alleges that one of ordinary skill in the art would recognize that amino acid biosynthetic pathways do not significantly vary between organisms, particularly bacterial strains, and therefore, one of ordinary skill in the art would have a reasonable expectation of increased amino acid yields by disruption of the *C. glutamicum pgi* gene. Applicants respectfully disagree with the Examiner. Amino acid biosynthetic pathways do, in fact, significantly vary between bacteria. Specifically, Sahm, at page 28, states:

> three different pathways of D,L-diaminopimelate and L-lysine synthesis are known in prokaryotes. All bacteria investigated in detail so far appear to use only one of the three pathways. For example, *Escherichia coli* is using the succinylase variant, whereas in *Bacillus subtilis* the acetylase variant is responsible for lysine synthesis. However, recently Schrumpf *et al.* have detected that in *C. glutamicum* the dehydrogenase variant and the succinylase variant exist side by side, both allowing D,L-aminopimelate and L-lysine synthesis. This is the only bacterium so far known that has two parallel anabolic pathways, their functions, besides the synthesis of D,L-diaminopimelate for cell wall synthesis and lysine synthesis, are not known.

Therefore, Sahm clearly shows that the lysine biosynthetic pathway differs significantly among bacteria. *E. coli* and *C. glutamicum* are specifically mentioned as using different biosynthetic pathways.

Therefore, it is inaccurate to state that one of ordinary skill in the art would have a reasonable expectation of increased amino acid yields by disruption of the *C. glutamicum pgi* gene based on the teaching in Mascarenhas. Sahm clearly teaches that there are differences in the biosynthetic pathways among bacteria, and simply because disruption in the *pgi* in *E. coli* resulted in greater lysine yield, it does not follow that disruption in the *pgi* in *C. glutamicum* will yield the same result. The Examiner is using impermissible hindsight in concluding that the invention is obvious as there is no suggestion or motivation to combine the references, and even if there was, there would be no reasonable expectation of success and the prior art references do not teach or suggest all of the claim limitations.

Ishino does not cure the deficiencies of Mascarenhas. Ishino investigates C. glutamicum glucose metabolism. Ishino indicates that the hexosemonophosphate (HMP) pathway contributes to lysine fermentation probably because of the greater requirement of NADPH in lysine formation from glucose. Ishino, however, does not teach the claimed invention. Specifically, Ishino does not teach a method of producing an amino acid selected from the group consisting of lysine, threonine and isoleucine by culturing a *C. glutamicum* with a disrupted *pgi* gene. Ishino, therefore, does not cure the deficiencies of Mascarenhas because it does not teach disrupting pgi gene to produce an amino acid.

Voet does not cure the deficiencies of Mascarenhas or Ishino. The Examiner states at page 6 of the Office Action that Voet teaches that the biosynthesis of lysine, threonine and isoleucine requires at least one molecule of NADPH. Voet, however, does not teach the

claimed invention because Voet does not teach a method of producing an amino acid selected from the group consisting of lysine, threonine and isoleucine by culturing a *C. glutamicum* with a disrupted *pgi* gene. Voet, therefore, does not cure the deficiencies of Mascarenhas because it does not teach disrupting pgi gene to produce an amino acid.

Sahm does not cure the deficiencies of Mascarenhas, Ishino or Voet. Sahm discusses some of the enzymes which contribute to lysine production in *Corynebacterium glutamicum.* Sahm, however does not teach or suggest culturing a *Corynebacterium glutamicum* cell, wherein the *C. glutamicum* cell has a disrupted *pgi* gene to produce lysine, threonine and isoleucine. Sahm describes disrupting enzymes involved in directing lysine production to yield strains which are feedback-resistant and thus produce more lysine. The claimed invention, however, relates to disrupting glucose metabolism in order to produce more NADPH, which is used in the production of lysine. Sahm is therefore fundamentally different in that Sahm describes directly disrupting the lysine pathway. Withdrawal of this rejection is therefore respectfully requested.

At page 8 of the Office Action, the Examiner has rejected claims 8 and 21 under 35 U.S.C. 103(a) as being unpatentable over Mascarenhas in view of Ishino, Voet, and Sahm as applied to claims 1, 6, 7 and 18-20 above and further in view of Fitzpatrick *et al.*, *Appl. Microbiol. Biotechnol. 42*:575-580 (1994) ("Fitzpatrick"). Applicants respectfully traverse this rejection.

Obviousness cannot be established absent some teaching, suggestion or incentive, and thus, even if it was obvious to one skilled in art to try various combinations to achieve the claimed methods (and Applicants do not believe it was), such evidence does not establish a *prima facie* case of obviousness. See *In re Geiger*, 2 USPQ2d 1276 (Fed. Cir. 1987) and

In re Fine, 5 USPQ2d 1596 (Fed. Cir. 1988). Furthermore, in order to establish obviousness, the Examiner must show that the prior art suggested the modification of the reference or references required to arrive at the claimed invention, and that the invention could be attained with a reasonable expectation of success. See *In re Vaeck*, 20 U.S.P.Q.2d 1438, 1442 (Fed. Cir. 1992).

As previously mentioned, Mascarenhas teaches the production of aromatic intermediates using *pgi*-deficient *Escherichia coli*. The present invention is directed to a method of producing L-lysine, L-threonine and/or L-isoleucine using *Corynebacterium glutamicum*, which is not disclosed in Mascarenhas *et al.* Fitzpatrick generally teaches only a method of subcloning a *recA* gene into the genome of *Corynebacterium glutamicum* for the purpose of gene silencing.

Therefore, Fitzpatrick does not cure the deficiencies of Mascarenhas *et al.* Fitzpatrick *et al.* is silent as to increasing the amounts of NADPH in *Corynebacterium glutamicum* to increase amino acid yields. Specifically, Fitzpatrick *et al.* does not teach or even suggest culturing *Corynebacterium glutamicum* cells with increased amounts of NADPH to produce the claimed amino acids. Therefore, the combination of these references does not render the invention obvious.

The Examiner again alleges at page 10 that "an ordinarily skilled artisan would have recognized that amino acid biosynthetic pathways do not significantly vary between bacterial strains, and therefore, one of ordinary skill in the art would have a reasonable expectation of increased amino acid yields by disruption of the *C. glutamicum pgi* gene. One of ordinary skill in the art would have applied the method of Fitzpatrick *et al.* for disruption the *C. glutamicum recA* gene in order to disrupt the *C. glutamicum pgi* gene."

Applicants respectfully disagree with the Examiner. As stated above, Sahm teaches that the biosynthetic pathways do significantly vary among bacteria; and specifically, there is significant variance in the *E. coli* lysine pathway versus the *C. glutamicum* lysine pathway. Fitzpatrick does nothing to cure the deficiencies present in Mascarenhas. Specifically, Fitzpatrick *et al.* does not teach or even suggest culturing *Corynebacterium glutamicum* cells with increased amounts of NADPH to produce the claimed amino acids.

For the foregoing reasons, the invention is not obvious in view of Mascarenhas *et al.*, and the addition of Fitzpatrick *et al.* does not add anything to render the invention unpatentable. Therefore, withdrawal of this rejection is respectfully requested.

Conclusion

All of the stated grounds of objection and rejection have been properly traversed, accommodated, or rendered moot. Applicants therefore respectfully request that the Examiner reconsider all presently outstanding objections and rejections and that they be withdrawn. Applicants believe that a full and complete reply has been made to the outstanding Office Action and, as such, the present application is in condition for allowance. If the Examiner believes, for any reason, that personal communication will expedite prosecution of this application, the Examiner is invited to telephone the undersigned at the number provided.

Prompt and favorable consideration of this Amendment and Reply is respectfully requested.

Respectfully submitted,

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Version with markings to show changes made

1. (Twice Amended) A method of producing an amino acid selected from the group consisting of L-lysine, L-threonine and L-isoleucine comprising:

culturing an altered *Corynebacterium glutamicum* cell [having an increased amount of NADPH as compared to an unaltered *Corynebacterium glutamicum* cell], wherein said *Corynebacterium glutamicum* cell has a disrupted pgi gene, wherein yields of an amino acid selected from the group consisting of L-lysine, L-threonine and L-isoleucine from said altered *Corynebacterium glutamicum* cell having a disrupted pgi gene are greater than yields from a [an unaltered] *Corynebacterium glutamicum* cell having a non-disrupted pgi gene.

6. (Twice Amended) The method of claim 1, wherein said L-amino acid yields from said [cultured] altered *Corynebacterium glutamicum* cell having a disrupted pgi gene are from about 1% to about 100% greater than from said [unaltered] *Corynebacterium glutamicum* cell having a non-disrupted pgi gene.

8. (Twice Amended) The method of claim 1, wherein said altered *Corynebacterium glutamicum* cell having a disrupted pgi gene is produced by

(a) subcloning an internal region of a *pgi* gene; and

(b) inserting said resulting vector from step (a) into a *Corynebacterium glutamicum* genome via homologous recombination.